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The Antioxidant and Antidepressant Properties of Dietary Proteins Derived from Egg and Bean Extracts and Their Acute Toxicity: A Journey from Nutrition to Pharmacognosy

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Article Information

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Keywords: Pharmacognosy; Antidepressant; Antioxidant; Acute toxicity; Eggs and beans

Abbreviations: i.p: Extracts Intraperitoneally; b.w.: Body Weight; DPPH: 2,2-diphenyl-1-picrylhydrazyl

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Abstract

This research reveals the previously unexplored pharmacognostic potential of antidepressants found in nutrients derived from both legume and animal sources. Through preclinical investigations involving mouse models, the study focused into antidepressant and antioxidant activities of non-denatured and denatured protein extracts from beans and eggs. Non-denatured protein extracts from beans and eggs, at saturation levels of 40% and 80%, were examined as macronutrients, while denatured protein extracts at equivalent saturation levels were considered micronutrients. The study employed the DPPH and hydrogen peroxide tests to assess antioxidant activity, and the forced swimming test and sucrose preference test to evaluate acute and chronic mild antidepressant effects, respectively. The acute toxicity study revealed that macronutrients from eggs at 40% and 80% saturation displayed non-toxic effects (LD50 >5 g/kg), while those from beans, specifically at saturation of 80%, exhibited a relatively low level of toxicity (LD50 = 2.5 g/kg). Evaluation of antioxidant activity using the DPPH test yielded inconclusive results due to the influence of ethanol precipitation. In contrast, the H_2O_2 test demonstrated significant antioxidant potential in both macronutrients and micronutrients extracted from beans and eggs at all saturation levels. In investigating antidepressant properties, both macronutrients and micronutrients of bean and egg protein extracts at 40% and 80% saturation exhibited notable antidepressant effects, particularly the micronutrients at saturation of 80%. This antidepressant effect was characterized by a reduction in immobility time and an increase in sucrose preference.

In conclusion, this study uncovers the multifaceted potential of protein extracts sourced from natural products, plant and animal origins, as agents for treating depression. It opens up new avenues for research, with implications ranging from neuroprotection to the management of depression, inspiring optimism for innovative approaches to mental health treatment.

Introduction

In recent years, the intricate connection between diet and mental health has garnered increasing attention within the realm of scientific inquiry. Among the various dietary components, proteins have emerged as crucial players not only in maintaining physical health but also in influencing mental well-being [1-3]. The impact of dietary proteins on the complex landscape of depression has become a subject of considerable

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interest, as researchers seek to uncover the potential benefits they may offer in mitigating depressive symptoms [1,2].

Depression, a prevalent and debilitating mental disorder, poses a significant global health burden. Its multifaceted etiology involves a combination of genetic, environmental, and neurochemical factors [4]. Traditional treatment approaches often involve psychotherapy and pharmacotherapy, yet the quest for innovative and holistic interventions has led researchers to explore the potential influence of diet on mental health outcomes [5]. In this context, proteins, the essential building blocks of life, have emerged as not only nutritional necessities but also as agents that might impact the brain's chemistry and functioning [6].

Proteins are instrumental in a multitude of physiological processes, including the synthesis of neurotransmitters, enzymes, and hormones [7]. These bioactive compounds play a pivotal role in regulating mood, cognition, and emotional well-being [8]. Recent studies have illuminated the intricate relationship between dietary protein intake and the intricate neural pathways that govern depression [2]. Amino acids, the foundational components of proteins, are essential precursors to neurotransmitters such as serotonin and dopamine, which are closely linked to mood regulation [9].

With the objective of achieving this aim, the current study was undertaken to investigate the antidepressant and antioxidant attributes of protein extracts sourced from beans and eggs. These extracts underwent analysis at saturation levels of 40% and 80%, with categorization as macronutrients in their undenatured state, and as micronutrients when subjected to denaturation.

Materials and methods

Materials

Food materials: The food materials used consist of eggs and beans. The materials were purchased from a grocery store.

Animal materials: *In vivo* experiments were carried out on both male and female mice. These mice have a weight ranging from 120g to 360g, and they have unrestricted access to water and food.

Methods

Preparation of protein extracts: Firstly, a bean macerate was prepared by adding 10g of bean powder to 100 mL of physiological solution (0.9% NaCl, pH 7.4). Then, the extracts were allowed to macerate for 24 hours at 4 °C. After maceration, the mixture was centrifuged (for 15 minutes at 2700 g at 4 °C) and the resulting supernatant was sterilized through a 0.45 µm nitrocellulose filter. In parallel, an egg extract was prepared by diluting egg white at a 1/12 ratio. Subsequently, the bean and egg protein extracts were prepared by adding ammonium sulfate at concentrations of 40% and 80% (w/v) at 4 °C [10]. Then, the samples were subsequently stored until needed.

Heat treatment: This process entails denaturing the proteins found in the protein extracts of eggs and beans. To accomplish this, protein extracts used to be heated for 30 minutes in a water bath maintained at 100 °C. In order to prevent any evaporation of sample water, the containers were hermetically sealed. After the treatment, the samples were cooled in a water bath maintained at 25 °C and then left at room temperature for about an hour before being stored in the cold room at 4 °C [11].

Acute toxicity study: We examined the acute toxicity of the egg and bean extracts in mice weighing between 120 and 360g. The mice were organized into seven groups of four each (n = 4; 2 male and 2 female). After administering the extracts intraperitoneally (i.p), ranging from 0.7 to 5g/Kg body weight (b.w.), we observed and recorded their overall behavior. At the culmination of the study, the animals were administered sodium pentobarbital for anesthesia, followed by euthanasia. Essential organs, including the liver, kidneys, spleen, lungs, and heart, were carefully removed, rinsed with a solution of 0.9% NaCl, and then weighed. The relative weight of each organ was determined using the subsequent formula [12]:

$$RW = (W_0/BW) \times 100$$

Where:

RW: Relative weight of the organ.

Wo: Weight of the organ in grams.

BW: Body weight of the rat in grams.

Antioxidant assays

- **DPPH assay**

  We evaluated the antioxidant potential of the examined extracts through the employment of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as outlined in the study by Qamar, et al. [13]. According to this procedure, various concentrations (5-200 µg/mL) of the tested extracts and ascorbic acid (used as a reference) were prepared. Subsequently, 1mL of each tested extract was combined with 3mL of pre-dissolved DPPH (0.004%) in ethanol. The mixture was thoroughly mixed and then allowed to incubate for 30 minutes at room temperature under light protection. Following this incubation period, the spectrophotometric measurement, using a Zuzi

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spectrophotometer, of the solutions' absorbance at 517 nm against a blank containing only ethanol was conducted. The inhibition percentage (I%) of the DPPH free radical was then calculated using the formula:

$$I\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$$

- **Hydrogen peroxide assay**

The antioxidant potential of the extracts using the H_2O_2 test was evaluated as described by Qamar, et al. [13]. A series of concentrations for the studied protein extracts (5-200 µg/mL) and ascorbic acid were prepared beforehand. Next, 1mL of the tested extracts was combined with 0.6mL of H_2O_2 (40 mM). Following this, the reaction mixture was incubated at room temperature for 10 minutes. Afterward, the absorbance of H_2O_2 was determined using a UV-Vis spectrophotometer (model 6300, VWR) at a wavelength of 230 nm. The percentage of H_2O_2 inhibition was calculated using the subsequent formula:

$$I\ (\%) = [(A_{blank}-A_{sample})/A_{blank}] \text{ x } 100$$

Forced swim test: This test was employed to investigate the antidepressant effects of protein extracts from eggs and beans on acute depression [14]. The forced swim test, a recognized behavioral assessment tool, was utilized as described by Porsolt, et al. [15]. Prior to the test, the mice were divided into twenty groups, with each group consisting of four animals (n = 4; 2 females and 2 males). Subsequently, the protein extracts were administered via "i.p" at various doses ranging from 14 µg/Kg (b.w.) to 140 µg/Kg (b.w.) for the test groups. The control groups were received a solution of 0.9% NaCl at a pH of 7.4, while the reference groups were treated with sertraline, a reference molecule known for its antidepressant properties. Following a 30-minute interval after extract administration, each mouse was placed in a water-filled chamber, maintaining a temperature between 25 °C and 27 °C. During the final four minutes of the test, the immobility time of each mouse was meticulously recorded. This immobility duration serves as a crucial indicator of behaviors associated with depression-like.

Sucrose preference test: This assay was employed to assess the antidepressant qualities of protein extracts sourced from eggs and beans in the context of chronic mild depression [14]. The sucrose preference test, a widely recognized behavioral evaluation method introduced by Willner [16], was implemented for this investigation. To execute the experiment, the protein extracts were administered "i.p" to the mice on a daily basis, encompassing doses ranging from 14 µg/Kg (b.w.) to 140 µg/Kg (b.w.). In order to streamline the testing procedure, the mice were organized into 16 distinct groups. Concurrently, control groups were subjected solely to a physiological solution (0.9% NaCl, pH = 7.4), while additional groups of mice were treated with sertraline, reference molecule. Subsequent to the extracts administration, the mice were individually housed in cages, each furnished with two separate bottles: one containing water and the other a 1% sucrose solution. The daily consumption of both water and the sucrose solution was methodically measured and recorded. To mitigate potential confusion, the positions of the two bottles were alternated daily. Calculation of sucrose preference was conducted using the following formula:

$$\text{Sucrose preference} = \frac{\text{Volume sucrose solution cousumed}}{\text{Total volume of liquide cousumed(water + sucrose solution)}} \times 100$$

Statistical analysis

The results acquired were presented as the mean ± standard error of the mean (SEM). To contrast the means among various groups, the Anova test was utilized. A finding was deemed statistically significant if the p - value was below 0.05 ($p < 0.05$).

Results

Acute toxicity study

Morphological and behavioral observations

- **Effects of undenatured bean protein extracts**

Table 1 displays the primary morphological and behavioral changes following the administration of undenatured protein extracts derived from beans at saturation levels of 40% and 80%, spanning a range of doses. Aside from the observed fur alterations and increased restlessness among the animals (Table 1), no further behavioral changes were detected throughout the 14-day duration of the study, even at the highest administered dose and saturation levels (5000 mg/Kg, b.w.; and 80% saturation).

- **Effects of undenatured egg protein extracts**

Following the administration of undenaturedegg protein extracts at saturation levels of 40% and 80% across various dosages, noticeable changes in the appearance of fur and heightened restlessness in the animals were recorded (Table 2). Specifically, some mice displayed raised fur spikes on the seventh day subsequent to receiving a dosage of 5000 mg/Kg (b.w.) of the 40% protein extract. Similar alterations in appearance were observed in the animals on the twelfth day of the study when administered a dosage of 700 mg/Kg (b.w.) of the same extract. Nevertheless,

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Table 1: Morphological and behavioral changes resulting from the administration of undenatured bean protein extracts.

Behavioral and physical changes	Negative control	Protein extracts at 40% of saturation			Protein extracts at 80% of saturation		
		700 mg/Kg	2000 mg/Kg	5000 mg/Kg	700 mg/Kg	2000 mg/Kg	5000 mg/Kg
Skin	-	-	-	-	-	-	
Hair	-	+	+	-	-	+	-
Somato-motor function	-	-	-	-	-	-	-
Agitation	-	+	+	+	+	+	+
Convulsion	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-
Sleep	-	-	-	-	-	-	-
Coma	-	-	-	-	-	-	-
Eyes stain	-	-	-	-	-	-	-
Breathing	-	-	-	-	-	-	-

-: Lack of change; +:Presence of change

Table 2: Morphological and behavioral changes of the animals following the administration of undenatured egg protein extracts.

Behavioral and physical changes	Negative control	Protein extracts at 40% of saturation			Protein extracts at 80% of saturation		
		700 mg/Kg	2000 mg/Kg	5000 mg/Kg	700 mg/Kg	700 mg/Kg	2000 mg/Kg
Skin	-	-	-	-	-	-	-
Hair	-	+	-	+	-	-	-
Somato-motor function	-	-	-	-	-	-	-
Agitation	-	+	+	+	+	+	+
Convulsion	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-
Sleep	-	-	-	-	-	-	-
Coma	-	-	-	-	-	-	-
Eyes stain	-	-	-	-	-	-	-
Breathing	-	-	-	-	-	-	-

-: Lack of change; +:Presence of change

no additional shifts in behavior were documented over the course of the 14-day study period, even in cases involving the most concentrated protein extract (80%) at the highest dosage (5000mg/Kg, b.w.) (Table 2).

Weight evolution

- **Effects of undenaturedbean protein extracts**

Table 3 presents the results pertaining to the weight changes of the animals following the administration of undenatured bean protein extracts at saturation of40% and 80%, across different dosages. Monitoring the weight modifications in the treated animals did not reveal any weight-related toxic effects when compared to the control group. Insignificant weight fluctuations, whether in terms of gain or loss, were observed throughout the two-week study period among all groups, including the control group. It is important to note that these weight variations are not associated with the bean extracts under investigation (Table 3).

- **Effects of undenatured egg protein extracts**

As shown in Table 4, the weight progression of the animals subjected to undenatured egg protein extracts showed no significant changes. When compared to the control group, there were no observed toxic effects related to the weight of these animals. This indicates that the administration of undenatured egg protein extracts did not result in any adverse weight-related outcomes.

Organ weights

- **Effects of undenatured bean protein extracts**

The results concerning the effects of undenatured bean protein extracts on the vital organs of the animals indicated no significant differences in the relative weights of these organs (Table 5). In comparison to the control group, undenatured bean protein extracts at saturation levels of 40% and 80% did not induced any toxic effects on the vital organs, even at the highest dose (5000 mg/Kg, b.w.) (Table 5).

- **Effects of undenatured egg protein extracts**

Table 6 displays the organ weights of the animals after the administration of undenatured egg protein extracts at saturation levels of 40% and 80% at